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(54) Title: CARBOXAMIDE DERIVATIVES AS SELECTIVE INHIBITORS OF PATHOGENS

(57) Abstract: The present invention relates to the use of carboxamide compounds as selective inhibitors of pathogens, particularly viruses and, more particularly, herpesviridae. Surprisingly, these compounds show reduced side effects in comparison with previous antiviral compounds. Thus, a novel method for preventing or treating infections by pathogens, particularly herpesviridae is provided.

Carboxamide derivatives as selective inhibitors of pathogens

Description

The present invention relates to the use of carboxamide compounds as selective inhibitors of pathogens, particularly viruses and, more particularly, herpesviridae. Surprisingly, these compounds show reduced side effects in comparison with previous antiviral compounds. Thus, a novel method for preventing or treating infections by pathogens, particularly herpesviridae is provided.

Human Cytomegalovirus (HCMV) is a highly species specific ß-herpesvirus. Primary infection of healthy children and adults is usually asymptomatic, with a minority of cases developing a mononucleosis-like syndrome. In contrast, congenital infection (U.S 0.2%-2.2% per live birth; approx. 40,000 per year) leads to several neurological defects in 10 to 15% of infected neonates. Immunocompromised patients are another group of hosts facing serious diseases caused by HCMV infection or reactivation of a persistent infection. Up to 40% of AIDS patients, for example, develop retinitis, pneumonitis, gastroenteritis or disseminated HMCV disease. Allograft recipients (20,000 allograft transplantations per year in US) are often infected (or superinfected) by virus from the transplanted organ. Clinical symptoms in the postransplant period include prolonged fever, leukopenia, thrombocytopenia, atypical lymphocytosis, elevated hepatic transaminases and decreased graft survival. In bone marrow transplantations, HCMV infection has been associated with high mortality rates (80-90% for untreated HCMV pneumonia), which have been reduced by newer antiviral agents to 10-20% (reviewed in Britt J.B. and Alford C.A., 1996, Cytomegaloviruses, pp.2494-2523. In B.N.Fields, D.M.Knipe, P.M.Howley (ed.) Fields Virology, Lippencott-Raven Pub., Philadelphia).

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Ganciclovir is available for intravenous and oral administration and as an implant in the case of retinitis. Toxicities include leukopenia and thrombocytopenia. Foscarnet (phosphonoformic acid) exhibits considerable renal toxicity and is only available in intravenous form, which is also true for Cidofovir. Although treatment of HCMV-induced disease has been substantially improved with these inhibitors of the viral DNA polymerase and preemptive or early antiviral therapy in transplant patients (Hebart, H.et al., Drugs, 1998, 55:59-72), there is room for improvement in the toxicity profile. Especially in the treatment of retinitis in AIDS patients, where CMV infection has to be controlled for long periods of time and replication will resume once Ganciclovir and Foscarnet are removed, new classes of drugs are needed with better oral bioavailability and activity against emerging Ganciclovir-and Foscarnet-resistant strains.

Leflunomide, an immunomodulatory drug used in rheumatoid arthritis, was previously found to inhibit HCMV replication in cell culture. The antiinflammatory and immunosuppressive properties of Leflunomide have been demonstrated in animal models of autoimmune disease and organ transplant rejection (Reviewed in J.Rheumatology (1998) 25:20, Agents Actions (1991) 32:10) and it was recently approved for use in rheumatoid arthritis in the US (Scrip (1998) (2370):22). The proposed basis for the antiproliferative action of Leflunomide, for example, in mitogen-stimulated human T-lymphocytes (JBC (1998) 273:21682), is the suppression of the de novo pathway of pyrimidine synthesis. The active metabolite of Leflunomide is a noncompetitive inhibitor of Dihydroorotate dehydrogenase (DHODH) (Biochem.Pharmacol.(1995) 50:861; J.Pharmacol.Exper.ther.(1995) 275:1043; JBC (1995) 270:22467, Eur.J.Biochem.(1996) 240:292; Biochemistry (1996) 35:1270), a mitochondrial enzyme which catalyzes the rate-limiting step in this biosynthetic pathway. Most research groups favor the inhibition of DHODH as the major mechanism for Leflunomide-induced cytostatic effects (cited in JBC 273, 21682), however, inhibition of tyrosine kinases by Leflunomide

has been demonstrated in animal models and proposed to be a second importantmode of action in immunosuppression (J.Immunol.(1997) 159:22; JBC (1995) 270:12398; Biochem.Pharmacol.(1996) 52:527, J.Immunol.159:167). Tyrosine kinase inhibition is not affected by the addition of uridine, while DHODH inhibition can be reversed by uridine. In the case of Brequinar Sodium, which is another inhibitor of DHODH and an antimetabolite tested for cancer therapy (Cancer Res.(1986) 46:5014, Cancer Chemother.Pharmacol.(1993) 32:64), the finding that only antiproliferative effectes were reversed by uridine led to a patent claiming the reduction of non-immunosuppressive side effects by coadministration of uridine (Williams J.W., Chong, A., Xu, X.: WO 98/13047 (1994)).

Surprisingly, it was found that carboxamide derivatives can selectively inhibit kinases from pathogens, particularly from herpesviridae such as HCMV, without significantly inhibiting DHODH. Particularly, these compounds show a pronounced reduction in HCMV replication but do not inhibit purified recombinant human DHODH in an in vitro assay. This surprising uncoupling of antipathogen efficacy and inhibition of the de novo pyrimidine synthesis suggests that the subject carboxamide derivatives are free of the side effects associated with the antiproliferative and immunosuppressive properties of prior art medicaments such as Leflunomide.

These compounds have been shown previously to inhibit platelet-derived growth factor receptor activity (WO 95/19169 Sugen, Inc.), to have antibacterial activity (US 3,303,201) and to inhibit HIV reverse transcriptase (WO 96/16675 Rega Institut). A selective activity of these compounds against kinases from pathogens such as bacteria, protozoa or viruses, particularly without undesired side effects, has not yet been described.

Thus, the present invention refers to the use of compounds of the general formulae (Ia), (Ib), (Ic) or (Id):

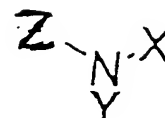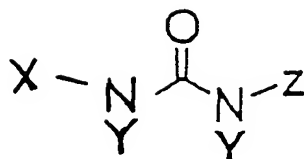(Ia)   (Ib)
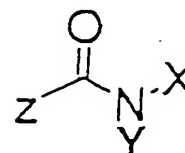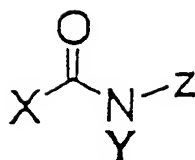
(Ic)   (Id)

wherein X and Z are substituents comprising an aromatic or heteroaromatic ring system for the manufacture of an agent against infectious diseases. For example, X and/or Z may be an aromatic radical, preferably a phenyl radical which is unsubstituted or which carries at least one substituent, e.g. 1-4 substituents which may be selected from hydroxy, cyano, nitro, halo, e.g. F, Cl, Br, I, C_1-C_4 alkyl, C_1-C_4 haloalkyl, C_1-C_4 alkoxy, C_1-C_4 haloalkoxy, C_1-C_4 alkylthio, C_1-C_4 haloalkylthio, carboxy, carboxy-C_1-C_4-alkyl, carboxy-aryl (or heteroaryl), amino carbonyl-C_1-C_4 alkyl, amino carbonyl - aryl (or heteroaryl) and aryl (heteroaryl), Y is hydrogen or C_1-C_4 alkyl.

Especially preferred are compounds, wherein X and/or Z is an aromatic or heteroaromatic radical, e.g. a phenyl radical having at least one, particularly one, two or three C_1-C_4 haloalkyl substituents, e.g. C_1 haloalkyl substituents such as -CF_3, -CHF_2 and -CH_2F.

For example, X and/or Z may be selected from radicals represented by formulae (IIa-c):
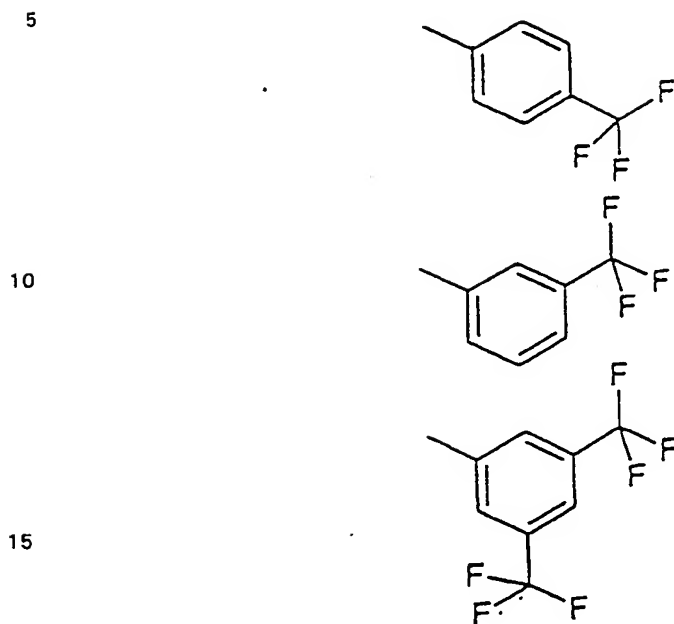
Y is preferably hydrogen or C_1-C_2 alkyl, more preferably hydrogen.

In a further preferred embodiment, X and/or Z comprises a heterocyclic ring which may contain one or several heteroatoms such as oxygen, nitrogen and/or sulfur, preferably a 5-membered heterocyclic ring which may be selected from pyrrole, pyrazole, imidazole, 1,2,3-triazole, tetrazole, oxazole, isoxazole, thiazole, isothiazole, 1,2,4-thiadiazole, 1,3,4-thiadiazole, thiophene, furan, indole and 3-thiaindole, a 6-membered heterocyclic ring which may be selected from pyridine, pyran, pyrimidine, pyridazine, pyrimidine and pyrazine, or a bi- or polycyclic heteroaromatic ring such as indazole, imidazole, chinoline or isochinoline. The heterocyclic ring can be mono, di, tri or tetrasubstituted with substituents as defined above.
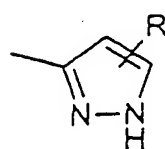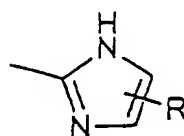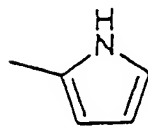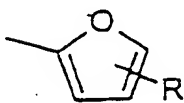
For example, X and/or Z may be selected from radicals represented by formulae (IIIa-k):
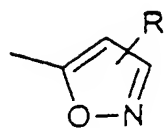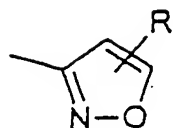
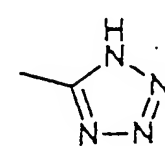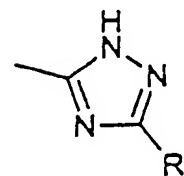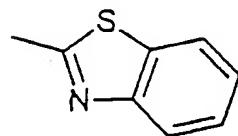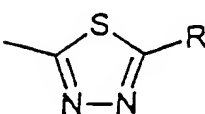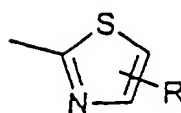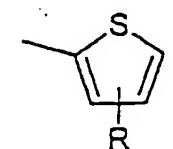wherein R is at least one substituent, e.g. one or two substituents selected from halo, C_1-C_3 alkyl, C_1-C_3 alkoxy or aryl, e.g. phenyl.

The aromatic and/or heteroaromatic ring of X an Z may be directly linked to the central structural element of the compounds (Ia-d), i.e. by a covalent

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bond. Alternatively, the linkage may comprise an alkylene-group, preferably a C_1-C_4 alkylene group, e.g. a C_1 or C_2 alkylene group.

Surprisingly, compounds (Ia), (Ib), (Ic) and (Id) have a selectivity for kinases from pathogens compared with the cellular enzyme DHODH. Thus, the compounds are suitable for the preparation of an agent against infectious diseases, particularly viral infections, more particularly, against infections by herpesviruses. The herpesviruses may be selected from human herpesviruses and herpesviruses from other mammals, such as bovine, equine, porcine and pongine herpesviruses. Suitable herpesviruses are selected from α-herpesviruses, e.g. simplexviruses such as herpes simplex virus 1, herpes simplex virus 2, bovine herpesvirus 2, cercopithecine herpesvirus 1 or varicellaviruses such as varicella zoster virus, porcine herpesvirus 1 (pseudorabiesvirus) bovine herpesvirus 1 and equine herpesvirus 1 (equine abortion virus). Further, the herpesvirus may be selected from ß-herpesviruses, e.g. cytomegaloviruses such as human cytomegalovirus and from roseoloviruses, such as human herpesvirus 6, human herpesvirus 7 or aotine herpesviruses 1 and 3. Further, the herpesviruses may be selected from γ-herpesviruses, e.g. from lymphocryptoviruses such as Epstein-Barr virus, cercopithecine herpesvirus 2 or porcine herpesvirus 1, or from rhadinoviruses such as human herpesvirus 8, ateline herpesvirus 2 or saimiriine herpesvirus 1, or preferably, the virus is selected from human herpesvirus 1 (HSV-1), varicella zoster virus (VZV) or human cytomegalovirus (HCMV).

Further, it was surprisingly found that the compounds are active against Foscarnet-resistant HCMV strains. Thus, they are likely to have a mode of action which is different from the HCMV drugs on the market, making them valuable tools for combination therapy approaches, e.g. for combination therapies together with viral DNA polymerase inhibitors. Furthermore, it was found that the compounds are potent inhibitors of ganciclovir-resistant virus strains, particularly ganciclovir-resistant HCMV strains.

The compounds of the formula I are suitable for the manufacture of an agent for the prophylaxis and/or treatment of a viral infection. This prophylaxis or treatment comprises administering a pharmaceutical composition containing as an active agent a pharmaceutically effective amount of a compound of the general formula I to a subject, preferably a human, in need thereof, e.g. a subject suffering from a herpesvirus infection or a subject which is in need of a prophylactic administration to avoid the outbreak of a herpesvirus infection. The pharmaceutical composition may contain suitable diluents, carriers and auxiliary agents. Further, the composition may also contain other pharmaceutically active agents, e.g. antiviral agents. The pharmaceutical composition may be suitable for oral, parenteral, e.g. intradermal, intravenous or intramuscular, rectal, nasal and topical applications. The composition may be an injectable solution, ointment, cream or spray. Further, the composition may have retardation properties, i.e. showing a delayed release of the active agent.

The dosage of the active agent depends on the specific compound being administered, the type and the severity of the viral infection. For example, a dosage from 0.01 mg to 100 mg per day and per kg/body weight for the active agent is suitable.

The efficacy of the compounds of formulae (Ia), (Ib), (Ic) and (Id) against enzymes from pathogens, e.g. viral kinases, may be determined in an in vitro enzyme inhibition test. On the other hand, the anti-viral effect can also be determined directly in a cell culture assay. A preferred and new cell culture assay is described in Example 2.2 and comprises the use of cells infected with a recombinant human cytomegalovirus carrying a reporter gene, e.g. the GFP-gene. The reporter gene is preferably inserted into the viral genome in a manner that viral replication is still possible. For example, the reporter gene may be inserted into the HCMV gene region encoding the open reading frames US9 and US10.

A further embodiment of the present invention refers to the use of compounds of the general formula (IV):

(IV)
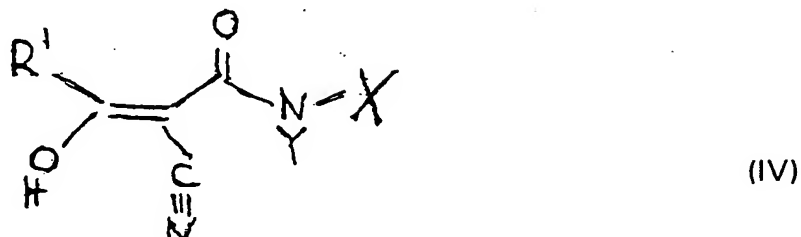
wherein X and Y are defined as for the compounds (Ia-d) and R' is selected from C_1-C_4 alkyl and C_3-C_4 cycloalkyl, particularly CH_3, for the preparation of an agent against ganciclovir-resistant virus strains. The compounds may be present in the form of a physiologically acceptable salt, e.g. an alkali metal, amonium or substituted amonium salt, particularly the sodium salt or the salt of a basic amino acid such as lysine. The compounds of formula (IV) may be classified as leflunomides. Surprisingly, these leflunomides are potent inhibitors of ganciclovir-resistant virus strains, particularly ganciclovir-resistant herpes virus strains and more particularly ganciclovir-resistant HCMV strains.

The compounds of the formula (IV) are suitable for the manufacture of an agent for the prophylaxis and/or treatment of a viral infection. This prophylaxis or treatment may be carried out as described above for compounds of the formulae (Ia) and (Ib).

Furthermore, the invention is to be explained by the following figures and examples.

Fig. 1   is a list of compounds of the present invention having anti-viral activity.

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Fig. 2   is a diagram showing the efficacy of compounds of the
            present invention against a ganciclovir-resistant virus strain.

Example 1

Synthesis of substituted anilides of heterocyclic carboxylic acids

A general procedure is given here suitable for acylation of substituted anilines with heterocyclic carboxylic acids. The aromatic rings can be mono, di, tri, or tetrasubstituted with e.g. alkyl, substituted alkyl, aryl, hydroxy, halogen, nitro, carboxy, thiomethyl groups. The heterocyclic ring can be mono, di, tri, or tetrasubstituted with e.g. alkyl, substituted alkyl, aryl, hydroxy, oxo, halogen, nitro, thiomethyl groups.

General procedure

1-10 mmol aniline was dissolved in dry 10-30 mol of dry pyridine and the solution was cooled to -20°C. 0.5 eq. of phosphorous trichloride was dropped in with vigorous stirring while the temperature was kept under -15°C. After 10-15 min the carboxylic acid was added slowly with stirring. The reaction mixture was allowed to warm to room temperature and stirred overnight, then warmed to 40°C until 30 min. Pyridine was removed in vacuum and the residue was taken up in 1N hydrochloric acid and ethyl acetate. The organic phase was washed with 5% hydrocarbonate solution and distilled water. After drying over sodium sulfate the solvent was evaporated and the residue purified via filtrating through silica layer, or chromatographed or recrystallized, if necessary.

All reagents and intermediates were purchased from Sigma-Aldrich.

For example, the following compounds were obtained:

(1)   Thiophene-2-carboxylic acid (3-trifluoromethyl phenyl)amide M.p.: 151-152°C R_f:0.73 (Ethylacetate:Hexane = 1:1)

(2)   Furane-2-carboxylic acid (3-trifluoromethyl phenyl)amide M.p.: 108-109°C R_f:0.73 (Ethylacetate:Hexane = 1:1)

(3)   Thiophene-2-carboxylic acid (3,5 bis-(trifluoromethyl) phenyl)amide M.p.: 148°C R_f:0.8 (Ethylacetate:Hexane = 1:1)

(4)   Furane-2-carboxylic acid (4-trifluoromethyl phenyl)amide M.p.: 108-109°C R_f:0.73 (Ethylacetate:Hexane)

(5)   Furane-2-carboxylic acid (3,5 bis-(trifluoromethyl) phenyl)amide M.p.: 124-125°C R_f:0.85 (Ethylacetate)

(6)   2-Cyano-3-hydroxy-crotonic acid (4-trifluoromethyl phenyl) amide ·

According to the general procedure as described above, further compounds of the formula I can be prepared, e.g. by using corresponding pyrrol, pyrrolidine or oxo-pyrrolidine carboxylic acids as starting materials. A list of such compounds is shown in Fig. 1.

Example 2

Anti-viral activity of compounds according to formulae (Ia) and (Ib)

2.1 DHODH assay

The cDNA for human DHODH (accession number M94065) was obtained via PCR with EST clone AA173225 (from RZPD in Berlin) and oligonucleotides 5'-CTG AAT TCA AAT TAC CGT GGA GAC ACC TGC AAA AGC GGG CCC AG-3' (this oligo provides the coding sequence for the

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first 5 amino acids missing in the EST clone) and 5'-AGC TCG AGT CAC
CTC CGA TGA TCT GCT CCA AT-3'. The PCR product was subcloned with
EcoRI and XhoI into vector pGEX 5x1 (Pharmacia, Gene (1988) 67:31) for
expression as a GST-fusion protein in bacteria. Recombinant GST-DHODH
was purified on glutathione-sepharose (method described in Gene (1988)
67:31) and dialyzed against 20 mM Tris pH 7.5, 0.1% Triton X100. The
assay was basically performed as described by Copeland et al. ("NBT
assay", Arch.Biochem.Biophys.323:79, 1995) with slight modifications: 20
μl of a 5x ubiquinone mixture (1 mM Ubiquinone Q10, 0.5% Triton X100,
500 mM Tris pH 7.5), 2 μl of 10 mM NBT (nitroblue tetrazolium), 2-10 μl
of DHODH-GST (depending on the batch of 1-liter bacterial preparation), 91-
83 μl of water and 5 μl of compound or DSMO were added to each well of
a 96-well microtiter plate. After mixing and incubation of the reactions for
5 min at 37°C, 5 μl of 10 mM L-dihydroorotate (or 5 μl of water in case of
the background controls) were added to start the reactions. After incubation
at 37°C for 60 to 90 min, ODs were read at 595 nm with a Biorad
microplate reader. Readings were in duplicate, controls without L-
dihydroorotate were performed as single measurements. Activity was
normalized with values from DMSO controls.

2.2 Virus Replication Assay

Cell culture and virus

Primary human foreskin fibroblasts (HFF) were cultivated in MEM containing
5% (v/v) fetal calf serum. Infection analysis was restricted to cell passage
numbers below twenty. Human cytomegalovirus strain AD169 (ATCC) was
grown in HFF cells and quantitated for infectivity by the plaque reduction
assay. Aliquots were stored at -80°C.

Construction of recombinant cytomegalovirus

For construction of a recombination vector, two linker sequences were
inserted into the pBlueScribe vector pBS + (Stratagene): the first contained

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restriction sites for NheI, SpeI, PacI and BglI followed by a loxP sequence (ATAACTTCGTATAGCATACATTATACGAAGTTAT) and was introduced into PstI/XbaI sites of the vector; the second contained another loxP sequence followed by restriction sites HpaI, ClaI and PmeI and was introduced into BamHI/Asp718 sites. A gene cassette comprising of a "humanized" version of the ORF coding for GFP (gfp-h) under the control of the HCMV enhancer/promoter and the Ptk/PY441 enhancer-driven neoR selection marker was excised from plasmid pUF5 (Zolotukhin et al., 1996, J.Virol.70, 4646-4654) and inserted into the recombination vector via BglII sites.

At the 5′ and 3′-positions of this loxP-flanked gene cassette, two HCMV sequences with homology to the gene region containing the open reading frames US9 and US10 were inserted. For this, viral sequences were amplified from template pCM49 (Fleckenstein et al., 1982, Gene 18, 39-46) via PCR in a 35-cycle program (denaturation 45 sec at 95°C, annealing 45 sec at 55°C and elongation 2 min at 72°C) by the use of Vent DNA polymerase (New England Biolabs). A US10-specific sequence of 1983 bp in length was generated using primers US10[200900]SpeI (GCTC**ACTAGT**GGCCTAGCCTGGCTCATGGCC) and US10[198918]PacI (GTCC**TTAATTAA**GACGTGGTTGTGGTCACCGAA) and inserted at the vector 5′ cloning position via SpeI/PacI restriction sites (see bold-print). A US9-specific sequence of 2010 bp was generated using primers US9-3′PmeI (CTCG**GTTTAAAC**GACGTGAGGCGCTCCGTCACC) and US-5′ ClaI (TTGC**ATCGAT**ACGGTGTGAGATACCACGATG) inserted at the vector 3′ cloning position via PmeI/ClaI restriction sites.

The resulting construct pHM673 was linearized by the use of restriction enzyme NheI and transfected into HEF cells via the electroporation method using a Gene Pulser (Bio-rad; 280 V, 960 μF, 400 Ω). After 24 h of cultivation, cells were used for infection with 1 PFU/ml of HCMV strain AD169. Selection with 200 μg/ml G418 was started 24 h post infection.

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Following 3 weeks of passage in the presence of G418, GFP fluorescence could be detected in most of the infected cells. Plaque assays were performed with infectuous culture supernatant on HFF cells and single virus plaques were grown by transfer to fresh HFF cells cultured in 48-well plates. DNA was isolated from cells of 32 fluorescence-positive wells and confirmed for the presence of recombinant virus by PCR. For this, primers US9[198789] (TGACGCGAGTATTACGTGTC) and US10[199100] (CTCCTCCTGATATGCGGTT) were used resulting in an amplification product of 312 bp for wild-type AD169 virus and approximately 3.5 kb for recombinant virus.

Plaque assay

HFF cells were cultivated in 12-well plates to 90-100% confluency and used for infection with dilutions of virus-positive cell culture supernatants. Virus inoculation was performed for 90 min at 37°C under occasional shaking before virus was removed and the cell layers were rinsed with PBS. Overlays of MEM 5% (v/v) fetal calf serum and 0.3% (w/v) agarose were added to each well and all samples were incubated at 37°C in a 5% CO_2 atmosphere for approximately 12 days. Finally, overlays were removed and the formation of foci was visualized by staining with 1% crystal violet in 20% ethanol for 1 min. After repeated rinsing with PBS, plates were air-dried at room temperature and plaque numbers were counted with a light microscope. For the recombinant AD169-GFP virus, quantification of plaque assays could also be performed without crystal violet staining by a direct counting of the amount of green fluorescent plaques using fluorescence microscopy.

Antiviral compounds

The reference compounds used for antiviral studies, ganciclovir (GCV, Cymeven), foscarnet sodium (FOS, Foscavir) and cidofovir (CDV, Vistide) were purchased from Syntex Arzneimittel (Aachen, Germany), Sigma-

Aldrich (Germany) and Pharmacia & Upjohn S.A. (Luxembourg), respectively. Stocks were prepared in aequeous solution and stored at -20°C. The test compounds were dissolved in DMSO and aliquots were stored at -20°C.

GFP infection assay

HFF cells were cultivated in 12-well plates to 90-100% confluency and used for infection with $0,5xTCID_{50}$ of AD169-GFP virus. Virus inoculation was performed for 90 min at 37°C with occasional shaking before virus was removed and the cell layers were rinsed with PBS. Infected cell layers were incubated with 2 ml of MEM containing 5% (v/v) fetal calf serum and optionally of the respective test substances or DMSO as control. Infected cells were incubated at 37°C in a 5% CO_2 atmosphere for 7 days and harvested by trypsination and centrifugation. 200 μl of lysis buffer (25 mM Tris pH 7.8, 2 mM DTT, 2 mM trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid, 1% Triton X-100, 10% glycerol) was added to each cell pellet and lysis was achieved by incubation for 10 min at 37°C followed by a 30-min incubation at room temperature on a shaker. Lysates were centrifuged for 5 min at 15.000 rpm in an Eppendorf centrifugue to remove cell debris. Supernatants were transferred to an opaque 96-well plate for automated measuring of GFP signals in a Victor 1420 Multilabel Counter (Wallac). GFP units were converted to percent inhibition values relative to DMSO controls (set at 100% GFP expression).

Indirect immunofluorescence analysis

Cells were either grown on Lab-Tek Permanox slides (Nunc) or harvested from 6-well plates, spotted onto glass slides with marked rings (Medco) and fixed by a 15-min treatment with 3% formaldehyde in PBS followed by permeabilization for 15 min in 0.1% Triton X-100 in PBS at room temperature. Blocking was achieved by incubation with Cohn Fraction II/III of human gamma-globulin (Sigma; 2 mg/ml) for 30 min at 37°C. The IE1/IE2-specific primary antibody MAb810 (Chemicon International, Inc. CA,

USA; dilution 1:10.000) was incubated for 90 min, the secondary antibody (tetramethyl rhodamine [TRITC]-coupled anti-mouse antibody, Dianova, dilution 1:100) for 45 min at 37°C before analysis by fluorescence microscopy. In addition to indirect TRITC staining of IE1/IE2 proteins, GFP

5   signals could be detected diretcly via the fluorescence isothiocyanate (FITC) channel. Nuclear counterstaining was carried out using Vectashield mounting medium including DAPI (Vector Laboratories, Burlingame, CA).

2.3 Results

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First, the ability of the compounds to inhibit the de novo pathway of pyrimidine biosynthesis, possibly leading to antiproliferative and immunosuppressive effects in vivo, was tested by determining DHODH activity in vitro in the presence of 20 μM of the test compounds. The

15   maximum inhibition of DHODH was 26%, whereas the active metabolite of Leflunomide inhibited at least 80% of DHODH activity under test conditions. Compounds (1) and (3), two compounds with a thiophene ring, were very effective in inhibiting HCMV replication at concentrations of 20 and 100 μM, while (2) which is identical to (1), except that it contains a furane ring

20   instead of a thiophene ring, showed slightly lower reduction at 20 μM concentration. Compounds (4) and (5) inhibited HCMV significantly at the higher, but not at the lower concentration.

Table 1 shows the compounds and lists which percentage of DHODH

25   activity is inhibited at a concentration of 20 μM. Values are averages of duplicate measurements from two different experiments. Percentage of HCMV inhibition at 20 μM and 100 μM (two different experiments) of the substances are also shown. Values are averages of duplicate measurements.

30

Table 1

	DHOD inhibition [%]	HCMV inhibition [%]	
Compound	20 μM	100 μM	20 μM
(1)	6 ± 10	99 ± 0	90 ± 4
(2)	5 ± 4	96 ± 1	73 ± 10
(3)	11 ± 9	100 ± 0	99 ± 0
(4)	12 ± 9	92 ± 1	9 ± 26
(5)	15 ± 11	98 ± 0	39 ± 12

The compounds shown in Fig. 1 also have significant anti-viral activity in the assay system as described above.

Example 3

Anti-viral activity against ganciclovir-resistant virus strains

3.1 Isolation of Drug-Resistant Virus

A series of laboratory variants of AD169-GFP virus with resistance against ganciclovir (GCV) was generated. HFF cells were infected in 12-well plates with MOI 0.002 and incubated with 1 μM of GCV. GFP-expression in infected cells was monitored microscopically and the supernatants of positive wells were transferred to fresh cells weekly. Thereby GCV concentrations were increased stepwise (1-μM increase in each step) up to the point where the total virus replication became critical and resistant virus grew out in individual wells. Using supernatants of these wells, two rounds of plaque purifications were performed on HFF cells. Finally, GCV-resistant

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viral clones (e.g. AD169-GFP314) were isolated which were able to replicate in the presence of 10 μM of GCV.

3.2 Virus Replication Assay

Plaque purification and plaque reduction assay

HFF cells were cultivated in 12-well plates to 90-100% confluency and used for infection with dilutions of virus stocks (i.e. AD169-GFP or AD169-GFP314). Virus inoculation was performed for 90 minutes at 37°C under occasional shaking before virus was removed and the cell layers were rinsed with PBS. Overlays of MEM containing 5% (v/v) fetal calf serum and 0.3% (w/v) agarose were added to each well. The plates were incubated at 37°C in a 5% CO_2 atmosphere for 8-12 d. For plaque purification of GFP-expressing viruses, plates were used for fluorescence microscopy, GFP-positive plaques were picked from the overlays and transferred to fresh cells for virus multiplication. For plaque reduction assays, antiviral compounds were incubated in the overlays after infection. Overlays were removed and plaque formation was visualized by staining with 1% cristal violet in 20% ethanol for 1 min. After repeated rinsing with PBS, plates were air-dried at room temperature and plaque numbers were counted with a light microscope. For the GFP-expressing recombinant viruses, quantification of plaque reduction assays could be performed alternatively, without cristal violet staining, by a direct counting of the numbers of green fluorescent plaques using fluorescence microscopy.

GFP-based antiviral assay

HFF cells were cultivated in 12-well plates (250,000 cells/well) and used for infection with 0.5xTCID$_{50}$-GFP of AD169-GFP or AD169-GFP314 virus. Virus inoculation was performed as described above. [TCID$_{50}$-GFP was defined as the dilution of the virus inoculum producing 50% of the maximal GFP signal in HFF cells]. Then, infected cell layers were incubated with 2,5 ml of MEM containing 5% (v/v) fetal calf serum and optionally a dilution of

one of the respective test compounds. Infected cells were incubated at 37°C in a 5% CO_2 atmosphere for 7 d. For lysis, 200 μl of lysis buffer (25 mM Tris pH 7.8 2 mM DTT, 2 mM trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid, 1% Triton X-100, 10% glycerol) was added to each well and incubated for 10 min at 37°C followed by a 30-min incubation at room temperature on a shaker. Lysates were centrifuged for 5 min at 15,000 rpm in an Eppendorf centrifuge to remove cell debris. 100 μl of the supernatants were transferred to an opaque 96-well plate for automated measuring of GFP signals in a Victor 1420 Multilabel Counter (Wallac).

3.3 Results

GCV-resistant HCMV is sensitive to compound (1) or compound (6).

HFF cells were cultivated in 12-well plates and infected with the indicated concentrations of GFP-expressing variants of HCMV, AD169-GFP (parental) or AD169-GFP314 (GCV-resistant). Immediately after the infection, the following chemical compounds were added to the culture media: DMSO 0.07% (A), 0.14% (B), ganciclovir (GCV) 10 μM (A and B), compound (6) 35 μM (A) and 70 μM (B), and compound (1) 35 μM (A) and 70 μM (B). Seven days postinfection infected cells were harvested and used for GFP quantification. The results for parental virus AD 169-GFP and a GCV-resistant virus mutant (AD169-GFP314) are shown in Fig. 2a and 2b, respectively.

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Claims

1.   Use of compounds of the general formulae (Ia), (Ib), (Ic) or (Id):
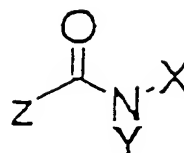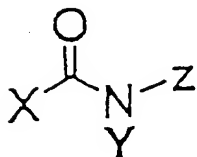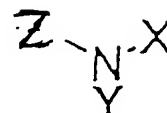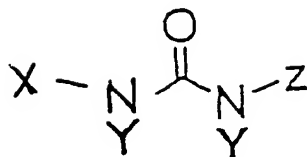
(Ia)   (Ib)

(Ic)   (Id)

wherein X and Z are substituents comprising an aromatic or heteroaromatic ring system and Y is hydrogen or C_1-C_4 alkyl as well as the pharmacologically acceptable salts thereof, for the preparation of an agent against infectious diseases.

2.   Use of claim 1,
wherein X and/or Z is an aromatic radical which is unsubstituted or which carries at least one substituent which may be selected from hydroxy, cyano, nitro, halo, C_1-C_4 alkyl, C_1-C_4 haloalkyl, C_1-C_4 alkoxy, C_1-C_4 haloalkoxy, C_1-C_4 alkylthio, C_1-C_4 haloalkylthio, carboxy, carboxy-C_1-C_4-alkyl, carboxy-aryl or -heteroaryl,

aminocarbonyl - C_1-C_4-alkyl, amino carbonyl - aryl or - heteroaryl, aryl and heteroaryl.

3.   Use according to claim 1 or 2,

wherein X and/or Z is a phenyl radical which may carry up to 4 substituents.

4.   Use according to claim 1 or 2,

wherein X and/or Z carries at least one C_1-C_3 haloalkyl substituent.

5.   Use according to claim 4,

wherein X and/or Z carries at least one substituent selected from -CF_3, -CHF_2 and -CH_2F.

6.   Use according to claim 5,

wherein X is selected from radicals represented by the formulae (IIa-c):
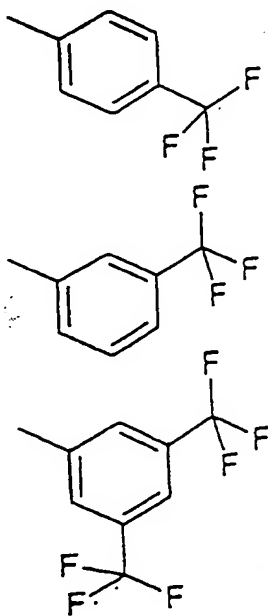
- 22 -

7.   Use according to any of the claims 1 - 6,
      wherein X and/or Z comprises a heterocyclic ring.

8.   Use according to any one of claims 1-5,
      wherein X and/or Z comprises a five-membered heterocyclic ring, a
      six-membered heterocyclic ring or a bi- or polyheterocyclic ring.

9.   Use according to claim 8,
      wherein X and/or Z comprises a five-membered heterocyclic ring
      selected from pyrrole, pyrazole, imidazole, 1,2,3-triazole, tetrazole,
      oxazole, isoxazole, thiazole, isothiazole, 1,2,4-thiadiazole, 1,3,4-
      thiadiazole, thiophene, furan, indole and 3-thiaindole.

10. Use according to claim 9,
      wherein X and/or Z is selected from radicals represented by the
      formulae (IIIa-k):
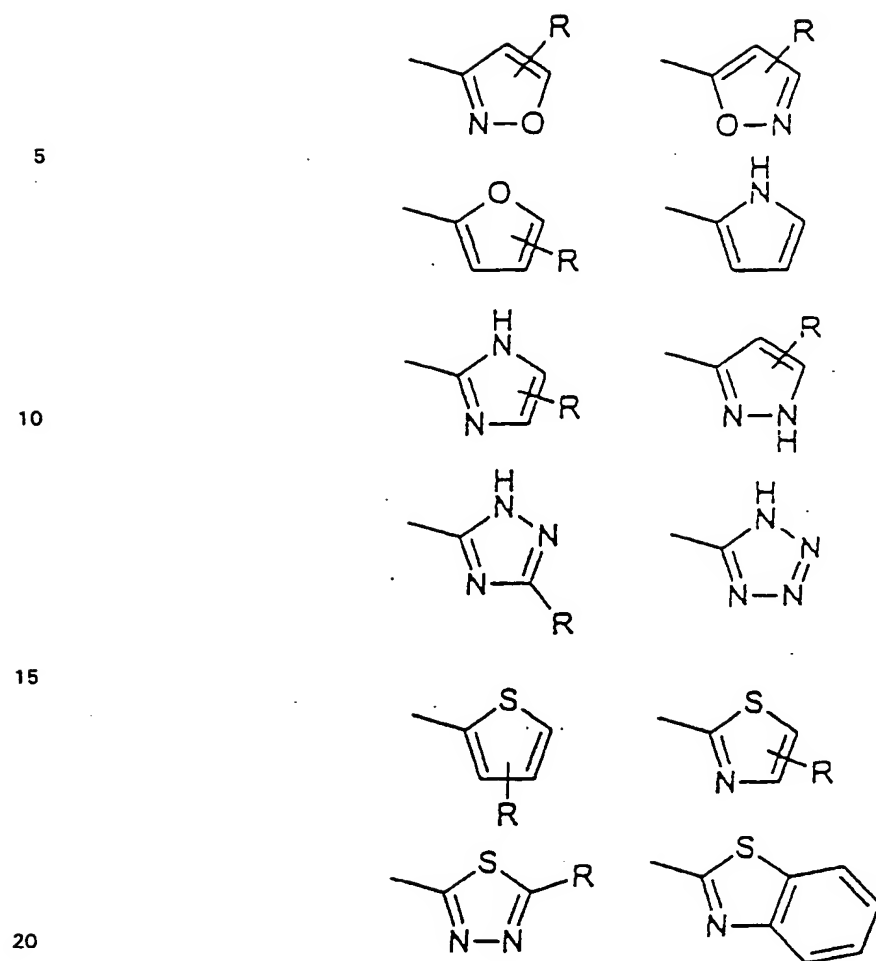
wherein R is at least one substituent selected from halo, C_1-C_3 alkyl and C_1-C_3 alkoxy.

11.   Use according to any one of claims 1-10,
       wherein compounds (Ia), (Ib), (Ic) and (Id) have a higher selectivity
       for kinases from pathogens than for the cellular enzyme DHODH.

12.   Use according to any one of claims 1-11 for the preparation of an
       agent against viral infections.

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13.   Use according to claim 12 for the preparation of an agent against infections by herpesviruses.

14.   Use according to claim 13,
         wherein the virus is selected from herpes simplex viruses, varicella viruses, cytomegaloviruses, muromegaloviruses, roseoloviruses, lymphocryptoviruses and rhadinovriuses.

15.   Use according to claim 14,
         wherein the virus is human cytomegalovirus (HCMV).

16.   Use according to any one of claims 12-15,
         wherein the virus is a Foscarnet- or ganciclovir-resistant virus strain.

17.   Use according to any one of claims 1-16,
         wherein the agent is suitable for oral, parenteral, rectal, nasal and topical application.

18.   Use according to any one of claims 1-17,
         wherein the dosage of the active agent is from 0.01 mg to 100 mg per day and per kg body weight.

19.   Use according to any one of claims 1-18 as a combination therapy with viral DNA polymerase inhibitors.

20.   A method for the treatment or prophylaxis of a herpesvirus infection comprising administering to a subject in need thereof a pharmaceutically effective amount of a compound according to general formulae (Ia), (Ib), (Ic) or (Id):
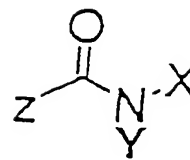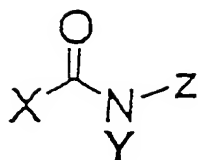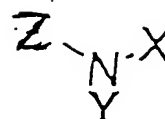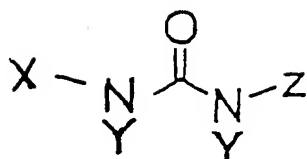
(la)   (lb)

X—C(=O)—N(Y)—Z

Z—C(=O)—N(Y)—X

(lc)   (ld)

X—N(Y)—C(=O)—N(Y)—Z

Z—N(Y)—X

wherein X and Z are substituents comprising an aromatic or heteroaromatic ring system and Y is hydrogen or C_1-C_4 alkyl.

21.   Compounds according to the general formulae (la), (lb), (lc) or (ld):
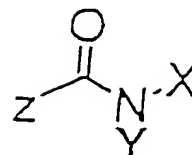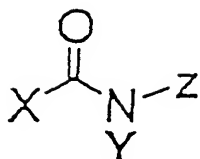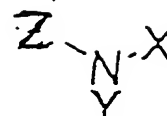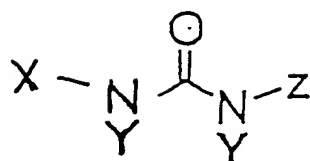
(Ia)   (Ib)

(Ic)   (Id)

wherein X and Z are substituents comprising an aromatic or heteroaromatic ring system and Y is hydrogen or C_1-C_4 alkyl.

22.   Use of compounds of the general formula (IV)

                                                    (IV)
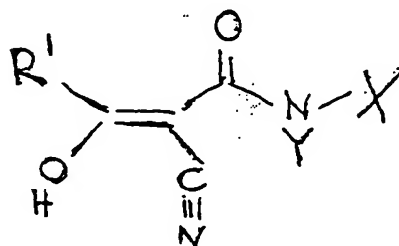
wherein X and Y are defined as in any one of claims 1-7 and R' is C_1-C_4 alkyl or C_3-C_4 cycloalkyl as well as the pharmacologically
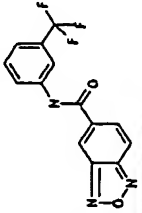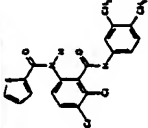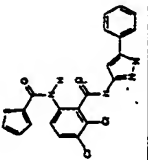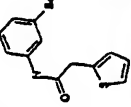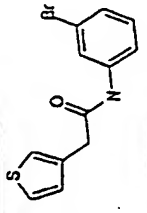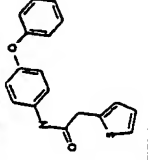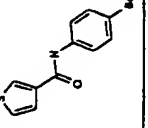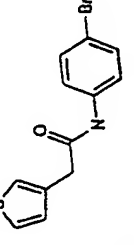
acceptable salts thereof, for the preparation of an agent against ganciclovir-resistant virus strains.

23.   Use according to claim 22,
       wherein R' is CH_3.

24.   Use according to claim 22 or 23,
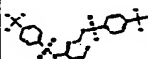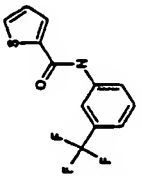   wherein the virus is human cytomegalovirus (HMCV).
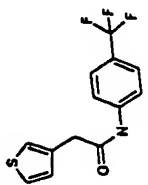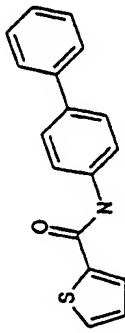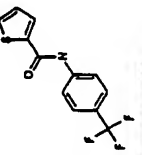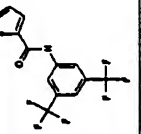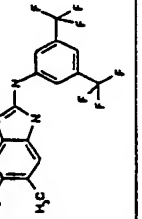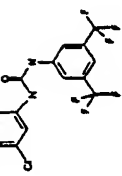
Fig. 1

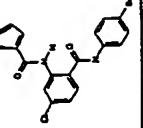| IUPAC NAME	STRUCTURE	MP	mol weigh	TLC Rf.
N-(5-Phenyl-1H-pyrazol-3-yl)-4-trifluoromethyl-benzamide		234 - 235	331,2999	0.59(B) 0.63(C)
4-Trifluoromethyl-N-(4-trifluoromethyl-phenyl)-benzamide		104 - 106	333,2355	0.79 (B) 0.51(C)
N-Quinolin-8-yl-4-trifluoromethyl-benzamide		75 - 78	316,2852	0.93(B) 0.51(C) 0.78(D)
N-(4-Iodo-phenyl)-4-trifluoromethyl-benzamide		148 - 151	391,1335	0.82(B) 0.49(C) 0.84(D)
N-(3-(4-trifluoromethyl-benzoyl)-amino-phenyl)-4-trifluoromethyl-benzamide		187 - 189	452,3595	0.61(B) 0.53(D)
N-(4-Cyano-phenyl)-4-trifluoromethyl-benzamide		179 - 180	290,247	0.64(B) 0.51(D)
3,4-Dimethyl-thieno[2,3-b]thiophene-2-carboxylic acid(bis-trifluoro methyl)-phenyl]amide		118 - 120	423,4017	Maybridge KM 03523
Quinoline-6-carboxylic acid (3-trifluoromethyl-phenyl)-amide		157 - 160	316,2852	KM 10124

continued Fig. 1
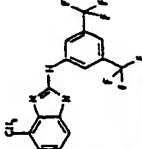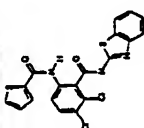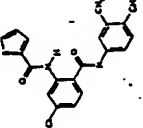
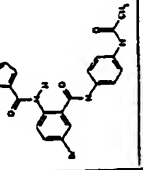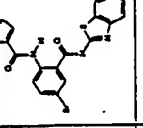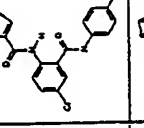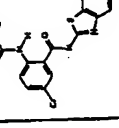| Name	Structure	mp (°C)	MW	Activity
Benzo[1,2,5]oxadiazole-5-carboxylic acid > (3-trifluoromethyl-phenyl)-amide		180 - 183	307,234 Maybridge KM 09572	
Thiophene-2-carboxylic acid[3,4-dichloro-2-(3,4-dimethyl)-phenyl]amide		222 - 223	419,3327	0.61(A) 0.67(B) 0.42(D)
Thiophene-2-carboxylic acid[3,4-dichloro-2-(5-phenyl-1H-pyrazol-3-ylcarbamoyl)-phenyl]-amide		254 - 255	457,3413	0.35(A) 0.50(B) 0.06(D)
N-(3-Bromo-phenyl)-2-thiophen-2-yl-acetamide		98 - 100	296,1876	0.56(A) 0.54(B) 0.36(D)
N-(3-Bromo-phenyl)-2-thiophen-3-yl-acetamide		100 - 101	296,1876	0.55(A) 0.55(B) 0.34(D)
N-(4-Phenoxy-phenyl)-2-thiophen-2-yl-acetamide		90 - 104	309,3898	0.52(A) 0.58(B) 0.32(D)
Thiophene-3-carboxylic acid (4-bromo-phenyl)-amide		194 - 196	282,1605	0.56(A) 0.51(B) 0.38(D)
N-(4-Bromo-phenyl)-2-thiophen-3-yl-acetamide		150	296,1876	0.54(A) 0.51(B) 0.29(D)

continued Fig. 1

Name	Structure	MP (°C)	Mass	Values
2-Thiophen-3-yl-N-(4-trifluoromethyl-phenyl)-acetamide		177 - 178	285,29	0.56(A) 0.50(B) 0.33(D)
Thiophene-2-carboxylic acid biphenyl-4-ylamide		203 - 204	279,3633	0.55(A) 0.55(B) 0.33(D)
N-(4-(4-trifluoromethyl-benzenesulfonyl)-amino-pyrimidin-2-yl)-4-trifluoro-methyl-phenylsulfonamide		175 - 176	526,4391	0.36(A) 0.48(B) 0.03(D)
Thiophene-2-carboxylic acid (3-trifluoromethyl-phenyl)-amide		128 - 130	271,2629	0.58(A) 0.46(B) 0.30(D)
Thiophene-2-carboxylic acid (4-trifluoromethyl-phenyl)-amide		170 - 172	271,2629	0.53(B) 0.35(D)
Thiophene-2-carboxylic acid (bis-trifluoromethyl-phenyl)-amide		145 - 146	339,2612	0.63(A) 0.47(B) 0.34(D)
(Bis-trifluoromethyl-phenyl)-(dimethyl-1H-benzoimidazol-2-yl)-amine		242 - 248	373,3037	0.57(A) 0.39(B) 0.14(D)
1-(Amino-dichloro-phenyl)-3-(bis-trifluoromethyl-phenyl)-urea		350	432,1549	0.56(A) 0.39(B) 0.10(D)

continued Fig. 1

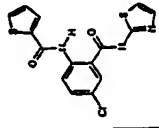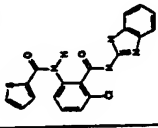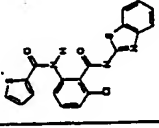| Compound	Structure	m.p.	MW	Rf
(3,5-Bis-trifluoromethyl-phenyl)-(4-methyl-1H-benzoimidazol-2-yl)-amine		232 - 235	359,2766	0.56(A) 0.40(B) 0.13(D)
Thiophene-2-carboxylic acid [2-(benzothiazol-2-ylcarbamoyl)-3,4-dichloro-phenyl]-amide		274 - 275	448,3524	0.52(A) 0.65(B) 0.18(D)
Thiophene-2-carboxylic acid[5-chloro-2-(3,4-dimethyl-phenyl-carbamoyl)-phenyl]amide		196 - 197	384,8877	0.61(A) 0.60(B) 0.31(D)
Thiophene-2-carboxylic acid [2-(4-bromo-phenylcarbamoyl)-5-chloro-phenyl]-amide		236 - 237	435,7295	0.61(A) 0.59(B) 0.29(D)
Thiophene-2-carboxylic acid [2-(4-acetylamino-phenylcarbamoyl)-4-bromo-phenyl]-amide		281 - 288	458,3368	0.35(A) 0.44(B) 0.02(D)
Thiophene-2-carboxylic acid [2-(benzothiazol-2-ylcarbamoyl)-4-bromo-phenyl]-amide		252 - 255	458,3584	0.56(A) 0.65(B) 0.21(D)
Thiophene-2-carboxylic acid [2-(4-bromo-phenylcarbamoyl)-4-chloro-phenyl]-amide		218 - 220	435,7295	0.61(A) 0.63(B) 0.34(D)
Thiophene-2-carboxylic acid [2-(benzothiazol-2-ylcarbamoyl)-4-chloro-phenyl]-amide		257 - 260	413,9074	0.57(A) 0.65(B) 0.25(D)

continued Fig. 1

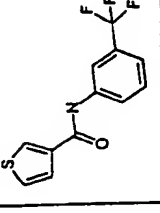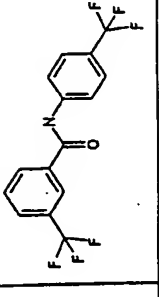| Name	Structure			
Thiophene-2-carboxylic acid [4-chloro-2-(thiazol-2-ylcarbamoyl)-phenyl]-amide		190	363,8469	0.51(A) 0.60(B) 0.13(D)
Thiophene-2-carboxylic acid[2-(1H-benzoimidazol-2-ylcarbamoyl)-3-chloro-phenyl]-amide		237 - 238	396,8581	0.35(A) 0.49(B) 0.03(D)
Thiophene-2-carboxylic acid [2-(benzothiazol-2-ylcarbamoyl)-3-chloro-phenyl]-amide		239	413,9074	0.47(A) 0.59(B) 0.13(D)
Thiophene-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide		119	271,2629	0.57(A) 0.47(B) 0.29(D)
3-Trifluoromethyl-N-(4-trifluoromethyl-phenyl)-benzamide		115 - 116	333,2355	0.62(A) 0.52(B) 0.34(D)

TLC conditions:
A: Hexane: Acetone 1:1
B: Benzene: Methanol 1:4
C: Ethylacetate: Hexane 1:5
D: Ethylacetate: Hexane 1:2

Fig. 2

(A) AD169-GFP (parental virus)

(B) AD169-GFP314 (GCV-resistant virus mutant)